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SHORT COMMUNICATION

DETECTION OF VIRAL CORES HAVING TOROID STRUCTURES IN EIGHT HERPESVIRUSES

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In cells infected with herpes simplex various shapes of viral core have been seen by electron microscopy (Morgan et al., 1959; Nii et al., 1968; Schwartz and Roizman, 1969). Such differences in morphology of the core were seen in observations on the intranuclear capsids of any given herpesvirus and differences in the fine structures of the core of different herpesviruses have also been reported (Nazerian et al., 1971; Nii, 1971a, 1971b). Recently, Furlong et al. (1972) reported that the core of the herpes simplex virion consists of an electron-dense toroidal structure penetrated by a less dense cylindrical mass and they suggested that the variation in the shape of the core observed in numerous studies may be explained by this toroid structure. More recently, Nazerian (1974) confirmed this three-dimensional structure of the core in Marek's disease virus.

We examined the ultrastructure of the core of eight herpesviruses. It was difficult to clarify the structure of the core by observations on enveloped particles of these viruses. However, various forms of core were seen in intranuclear particles and it was possible to classify them into several groups (Nii and Yasuda, in preparation). Moreover, cores with toroid structures were seen on infection with all the herpesviruses examined. This paper describes these cores.

The eight herpesviruses examined, including 5 human herpesviruses were type 1 and 2 herpes simplex virus, varicella-zoster virus, human cytomegalovirus, EB virus, Marek's disease virus, a herpesvirus of trukeys and herpesvirus cuniculi (Nesburn, 1969). To make samples for electron microscopy of all these viruses except EB virus, infected cells and the corresponding control normal cells were scraped into an appropriate amount of MEM and centrifuged at low speed. With EB virus, a cell suspension of the P3HR-1 strain (Hinuma et al., 1967) was centrifuged. The resulting pellets were fixed for 1 hr in 1% glutaraldehyde, washed well, fixed for 30 min in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Sections were made with a glass knife in an LKB microtome and stained with uranylacetate and lead citrate. A Hitachi 11B electron microscope was used.

Figs. 1-a, 1-b and 1-c show the intranuclear capsids of type 2 herpes simplex virus. In Fig. 1-a two viral capsids are seen. The core of one consists of an electron-dense torus, penetrated by a less electron-dense cylindrical mass. Both ends of this central cylindrical structure connect with the inner surface of

the capsid. Fig. 1-b illustrates two such capsids which are located in the fluffy area of low density next to the condensed chromatin. In one of the capsids shown in Fig. 1-c there is a central electron-dense torus which is perpendicular to the axis of a cylindrical mass. Neither end of this cylindrical structure seems to be directly connected with the capsid and this cylindrical mass seems to be held in the capsid by less dense material which appears to radiate from both its ends. Figs. 1-d and 1-e illustrate intranuclear capsids of type 1 herpes simplex virus. Each capsid has an electron-dense structure presumably representing a torus seen at an angle. In the capsid in Fig. 1-e there is a less electron-dense cylindrical mass, but this structure is not so clear in the capsid in Fig. 1-d. Capsids with a torus structure were also seen in cells infected with EB virus, as shown in Fig. 1-f. The capsids described above all had a single torus, but the capsid of EB virus shown in Fig. 1-g (arrow) seems either to have two tori or to have electron-dense material which is wound spirally around the cylindrical mass. Figs. 2-h and 2-i show intranuclear capsids of human cytomegalovirus. As seen in one of the capsids enclosing a toroid structure in Fig. 2-h (arrow), the cylindrical structures formed with this virus were usually slightly larger in diameter than those seen with other herpesviruses. The capsid seen in the center of Fig. 2-i is very close to an intranuclear electron-dense mass which occupies the left one third of the picture. In this capsid there are three electron dense toroid structures around a cylindrical mass, of which one appears to be thick and other two are filamentous in structure placed little apart on either side of the former thick dense structure. Fig. 2-j shows a capsid of varicella-zoster virus containing a toroid structure. Intranuclear capsids of Marek's disease virus are illustrated in Figs. 2-k, 2-l, 2-m and 2-n. Some capsids have cores like those reported by Nazerian (1974) (Fig. 2-k, 2-l), while other capsids have two tori or an electron-dense spiral structure (Fig. 2-m, 2-n). The herpesvirus of turkeys is not exceptional in producing capsids with a torus-like structure, although a high percentage of the intranuclear particles of this agent have an inner structure resembling an electron lucent cross (Nazerian et al., 1971; Okada et al., 1972). In the capsid indicated by an arrow in Fig. 3-o, an electron-dense torus-like structure surrounds a central, less dense cylindrical mass and two electron-dense filamentous structures, located slightly above and below the torus-like structure, are seen around the cylindrical mass. It seems possible that the three electron-dense structures observed in this capsid may be connected and comprise a single spiral structure around a cylindrical mass. Figs. 3-p, 3-q and 3-r show intranuclear capsids with a toroid structure found in HC-infected cells. The capsid in Fig. 3-q encloses a core with a typical form of a torus and a cylindrical mass. The core illustrated in Fig. 3-r probably represents a structure attached to the ends of a cylindrical mass similar to that presented in Fig. 1-c.

Thus, a type of core which consists of a torus and a penetrating cylindrical mass could be observed with all eight herpesviruses examined.

Furlong et al. (1972) reported that the core of the herpes simplex virion is a torus 50 nm long, with an inner diameter of 18 nm and outer diameter of 70 nm. The tori reported here were smaller. This must be because they were enclosed in naked, intranuclear capsids. It was rather difficult to clarify the spatial configuration of viral cores or to detect torus structures in enveloped particles. Therefore, we could not demonstrate that all virions of herpesviruses consist of a torus and a cylindrical mass.

Time-sequential studies on the appearance of various forms of viral core of type 2 herpes simplex virus are now in progress. The data so far obtained suggest that the toroid structure found in some intranuclear capsids represent a developmental stage of herpes virions. At present, however, it is still

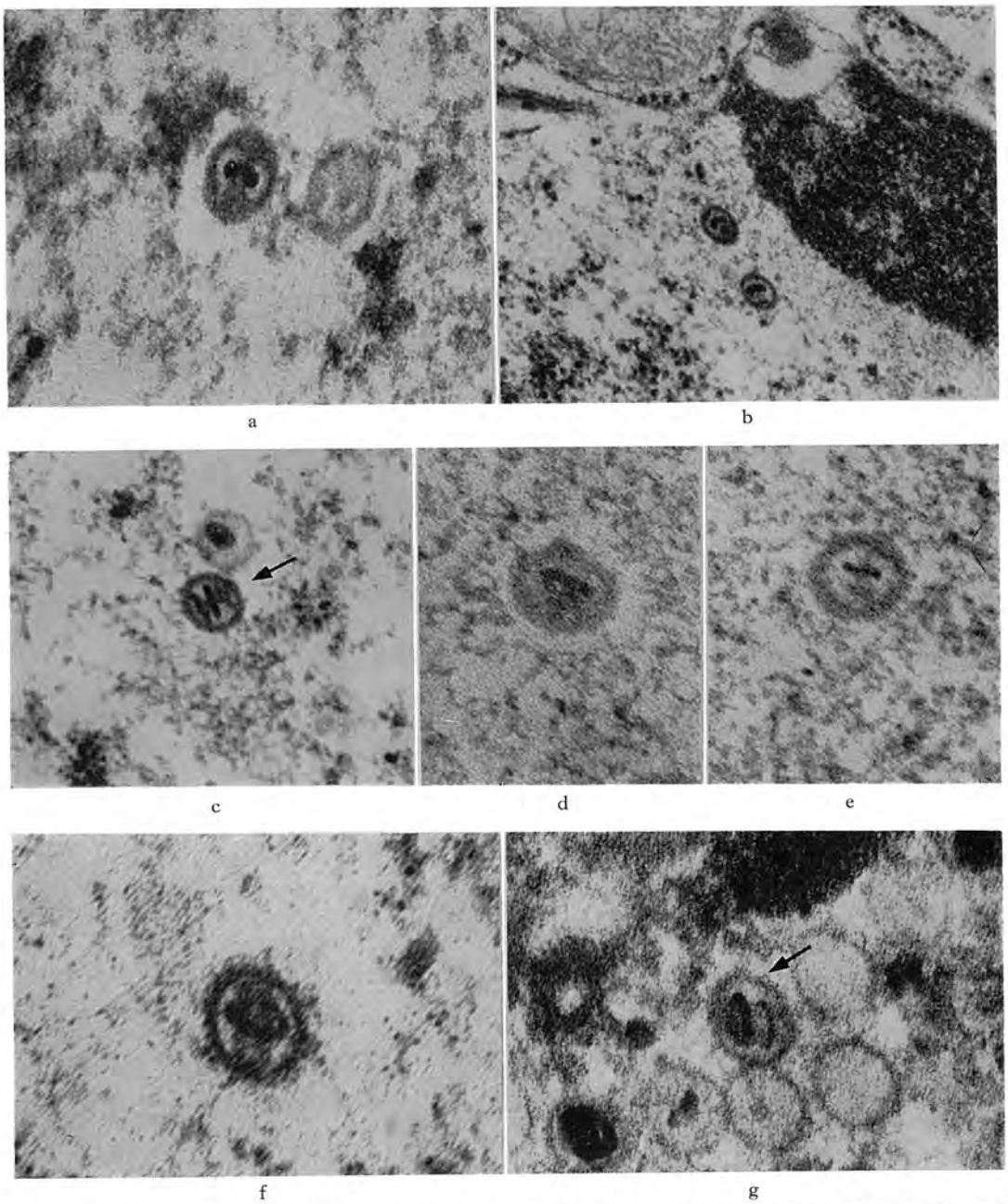

FIGURE 1. *Electron micrographs of intranuclear particles of type 1 and 2 herpes simplex and EB viruses. Capsids with a torus and a penetrating cylindrical mass are shown in Figs. 1-a~1-f and one capsid indicated by an arrow in Fig. 1-g has two tori or an electron-dense filamentous spiral structure wound around a cyrindrical mass. Figs. 1-a~1-c (type 2 herpes simplex virus), Figs. 1-d, 1-e (type 1 herpes simplex virus), Figs. 1-f, 1-g (EB virus). Magnification: Fig. 1-a, ×120,000; Fig. 1-b, ×60,000; Fig. 1-c, ×87,000; Figs. 1-d~1-f, ×130,000; Fig. 1-g, ×125,000.*

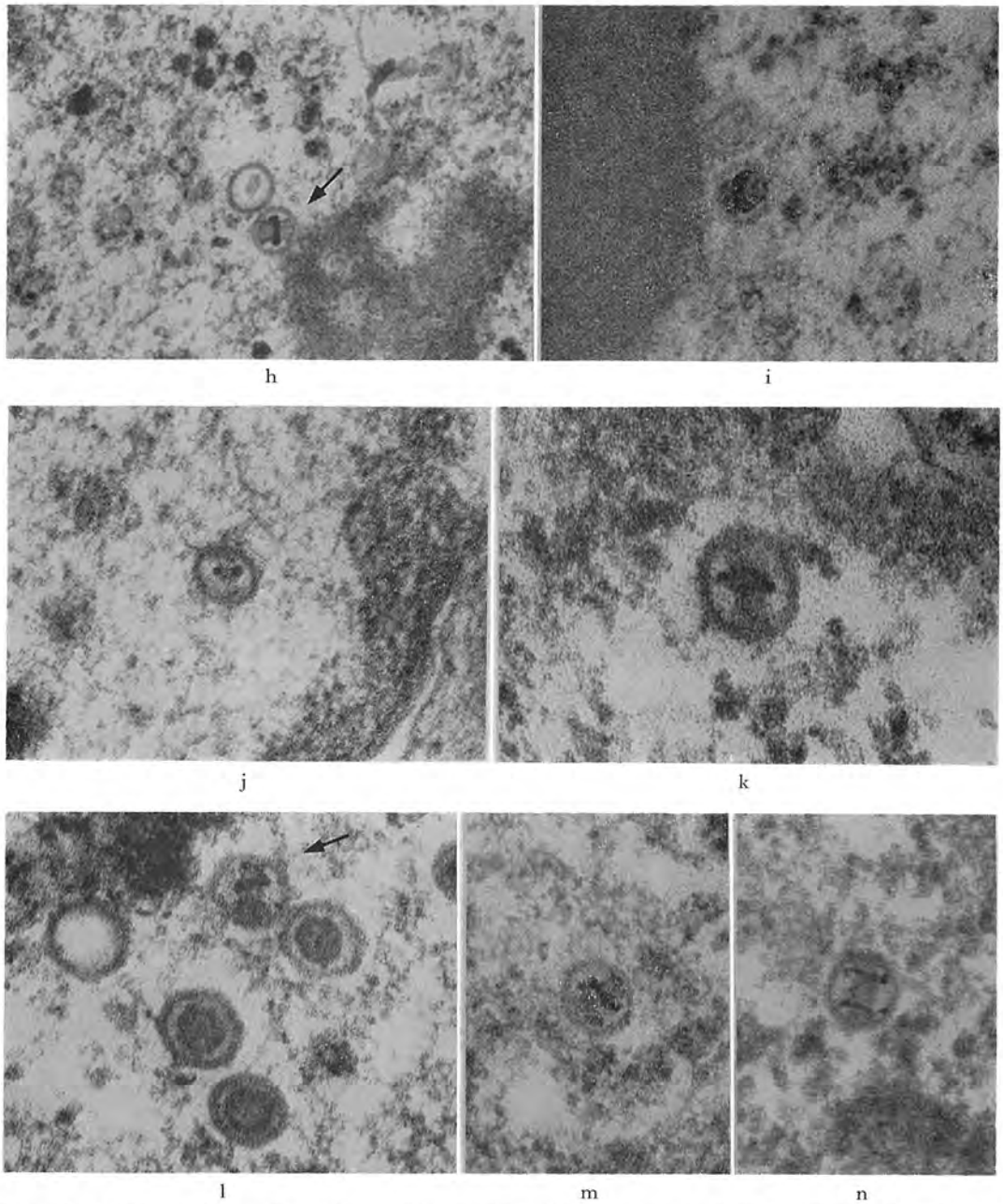

FIGURE 2. *Electron micrographs of intranuclear particles of human cytomegalovirus, and varicella-zoster and Marek's disease viruses. Capsids enclosing a torus structure are shown in Figs. 2-h, 2-j, 2-k and 2-l. Capsids containing two or more tori or an electron-dense spiral structure are shown in Figs. 2-i, 2-m and 2-n. Figs. 2-h, 2-i (human cytomegalovirus), Fig. 2-j (varicella-zoster virus), Figs. 2-k~2-n (Marek's disease virus). Magnification: Fig. 2-h, ×62,000; Fig. 2-i, ×80,000; Fig. 2-j, ×100,000; Fig. 2-k, ×150,000; Fig. 2-l, ×102,000; Figs. 2-m, 2-n, ×103,000.*

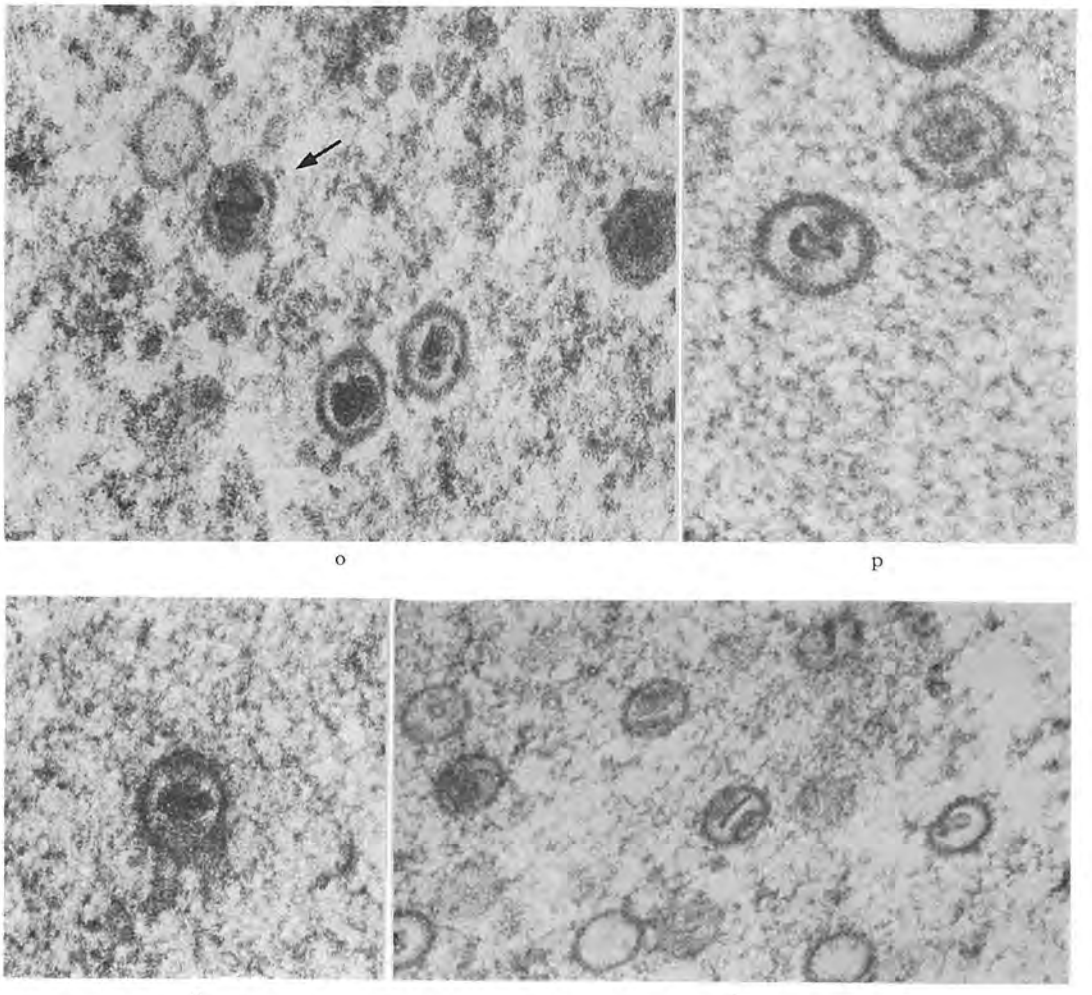

FIGURE 3. *Electron micrographs of intranuclear particles of a herpesvirus of turkeys and herpesvirus cuniculi. Capsids enclosing an electron-dense torus or a filamentous spiral structure of similar density (arrow) are shown. Fig. 3-o (a herpesvirus of turkeys), Figs. 3-p~3-r (herpesvirus cuniculi). Magnification: Fig. 3-o, ×117,000; Fig. 3-p, ×145,000; Fig. 3-q, ×130,000; Fig. 3-r, ×93,000.*

questionable whether the viral DNA in all cores of herpes virions is wound around a cylindrical structure connecting the two inner poles of the capsid. This problem will be discussed again in a subsequent report.

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